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Sirolimus-Eluting Stents versus Standard Stents in Patients with Stenosis in a Native Coronary Artery

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ABSTRACT

BACKGROUND

Preliminary reports of studies involving simple coronary lesions indicate that a sirolimus-eluting stent significantly reduces the risk of restenosis after percutaneous coronary revascularization.

METHODS

We conducted a randomized, double-blind trial comparing a sirolimus-eluting stent with a standard stent in 1058 patients at 53 centers in the United States who had a newly diagnosed lesion in a native coronary artery. The coronary disease in these patients was complex because of the frequent presence of diabetes (in 26 percent of patients), the high percentage of patients with longer lesions (mean, 14.4 mm), and small vessels (mean, 2.80 mm). The primary end point was failure of the target vessel (a composite of death from cardiac causes, myocardial infarction, and repeated percutaneous or surgical revascularization of the target vessel) within 270 days.

RESULTS

The rate of failure of the target vessel was reduced from 21.0 percent with a standard stent to 8.6 percent with a sirolimus-eluting stent ($P<0.001$) — a reduction that was driven largely by a decrease in the frequency of the need for revascularization of the target lesion (16.6 percent in the standard-stent group vs. 4.1 percent in the sirolimus-stent group, $P<0.001$). The frequency of neointimal hyperplasia within the stent was also decreased in the group that received sirolimus-eluting stents, as assessed by both angiography and intravascular ultrasonography. Subgroup analyses revealed a reduction in the rates of angiographic restenosis and target-lesion revascularization in all subgroups examined.

CONCLUSIONS

In this randomized clinical trial involving patients with complex coronary lesions, the use of a sirolimus-eluting stent had a consistent treatment effect, reducing the rates of restenosis and associated clinical events in all subgroups analyzed.

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THE DEMONSTRATED CLINICAL USEfulness of the implantation of a coronary stent as the preferred method of percutaneous revascularization is due to improved procedural safety as compared with balloon angioplasty and reduced rates of restenosis.[1-7] But despite the use of coronary stents, the frequency of restenosis may be more than 30 percent in several subgroups of patients, including subgroups with diabetes mellitus, small coronary vessels, and long lesions.[8-15]

During the past two decades, attempts to reduce restenosis after angioplasty with the use of locally delivered or systemic pharmaceutical agents have been largely unsuccessful.[16-19] Recently, sirolimus (rapamycin), a cytostatic macrocyclic lactone with both antiinflammatory and antiproliferative properties,[20-22] delivered from a polymer-encapsulated stent was shown in small registry studies and randomized clinical trials to reduce the risk of restenosis in patients who were at low risk for restenosis.[23-25] We conducted a study to determine the clinical usefulness of the sirolimus-eluting stent in patients with more challenging coronary stenoses.

METHODS

STUDY DESIGN AND ELIGIBILITY

This randomized, double-blind study complied with the provisions of the Declaration of Helsinki regarding investigation in humans and was approved by the Food and Drug Administration. The study was approved by the institutional review boards at all 53 investigational sites, and written informed consent was obtained from all patients.

Eligible patients had a history of stable or unstable angina and signs of myocardial ischemia. A single newly diagnosed target lesion in a native coronary artery resulting in stenosis of 51 to 99 percent of the luminal diameter and measuring 15 to 30 mm in length (as estimated visually on angiography) was treated. Major criteria for exclusion were recent myocardial infarction (within the previous 48 hours); an ejection fraction of less than 25 percent; a target lesion in an ostium, a bifurcation, or an "unprotected" left main coronary artery or in a vessel with thrombus or severe calcification; and treatment of nontarget lesions in the same or a different coronary vessel during the index procedure.

Before the index procedure, an automated telephone randomization system was used to randomly assign eligible patients in a double-blind manner to treatment with a sirolimus-eluting stent or a standard stent (Bx Velocity, Cordis) in a 1:1 ratio at each site. Randomization blocks were created and were stratified according to the clinical center and the presence or absence of diabetes mellitus.

CORONARY-STENT PROCEDURE

Before and after the index procedure, all patients received oral aspirin (325 mg daily) and oral clopidogrel (a loading dose of 300 to 375 mg 24 hours before the procedure and then 75 mg daily for three months). During the procedure, intravenous heparin boluses were administered. The use of intravenous glycoprotein IIb/IIIa inhibitors was at the discretion of the physician. Lesions were treated with the use of standard interventional techniques, including mandated balloon dilation before the placement of the stent. One or two stents of the assigned type were used to treat the target lesion. The sirolimus-eluting stents and the standard stents (available in lengths of 8 mm and 18 mm and in diameters of 2.5 mm, 3.0 mm, and 3.5 mm) were identical in appearance. The sirolimus-eluting stents contained 140 μg of sirolimus per square centimeter of stent-surface area within a copolymer matrix that was 5 to 10 μm thick and was designed to release approximately 80 percent of the total dose of sirolimus in 30 days. Both the physician and the patient were unaware of the treatment-group assignment.

DATA COLLECTION, FOLLOW-UP, AND CORE LABORATORY ANALYSES

All data were submitted to a data coordinating center (the Cardiovascular Data Analysis Center, Harvard Clinical Research Institute, Harvard Medical School, Boston), and the investigators had full access to the data. The investigators also initiated, performed, and reviewed all analyses and made the decisions about publication. Clinical follow-up information was obtained for all patients by the research coordinators at each site at 30, 90, 180, and 270 days. All clinical end points were adjudicated by an independent clinical-events committee that was unaware of the treatment-group assignments. A separate data and safety monitoring board that was not affiliated with the study sponsor or the investigators reviewed all data periodically to identify potential safety issues (all complications, including death, stent thrombosis, and myocardial infarction) and to review the conduct of the study (the pace of enrollment, patients' eligibility, and compliance with data collection). The monitoring board did not perform an interim analysis with regard to the pri-

mary efficacy end point at nine months, since enrollment was completed before the nine-month primary end point was reached in the first patient.

Coronary angiograms, obtained at base line, at the completion of the stenting procedure, and at 240 days of follow-up, were submitted to the angiographic core laboratory (Brigham and Women's Angiographic Core Laboratory, Boston) and were analyzed with the use of a computer-based system (Medis). "Binary" restenosis was defined as stenosis of more than 50 percent of the luminal diameter in the target lesion. Late luminal loss was defined as the difference between the minimal luminal diameter at the completion of the stenting procedure and that measured during follow-up. Quantitative angiographic measurements of the target lesion were obtained in the "in-stent" zone (including only the stented segment) and in the "in-segment" zone (including the stented segment as well as the margins 5 mm proximal and distal to the stent).

Intravascular ultrasonographic examinations were performed after the index stenting procedure and at 240 days in a subgroup of 250 consecutive patients at 17 centers. With the use of intravascular ultrasonography, qualitative assessments and quantitative determinations of the areas and volumes of the vessels, stents, and lumens were made by the intravascular ultrasonography core laboratory (Cardiovascular Core Analysis Laboratory, Stanford University, Stanford, Calif.).

STUDY END POINTS

The primary end point of this study was failure of the target vessel, defined as the occurrence of any of the following within 270 days after the index procedure: death from cardiac causes, Q-wave or non–Q-wave myocardial infarction, or revascularization of the target vessel (emergency or elective coronary-artery bypass grafting [CABG] or repeated percutaneous transluminal coronary angioplasty [PTCA]).

The secondary clinical end points included death from any cause, revascularization of the target lesion (clinically driven CABG or repeated PTCA due to restenosis or closure of the target lesion), and stent thrombosis. All major adverse events were determined for the in-hospital period, for the out-of-hospital period, and cumulatively for the 270 days after the placement of the stent.

STATISTICAL ANALYSIS

The planned sample size of 1100 patients provided 80 percent statistical power to detect a 40 percent reduction in the rate of the primary end point at 270 days (from 15 percent with the standard stent to 9 percent with the sirolimus-eluting stent) with a 5 percent false positive rate (two-sided). We prespecified that the effectiveness analysis and the safety evaluation were to be based on data from all patients who underwent randomization except those who were withdrawn before they received the assigned treatment (as described below). The differences between the treatment groups were evaluated with the use of analysis of variance or with Wilcoxon rank-sum scores for the continuous variables, when appropriate. The Cochran–Mantel–Haenszel statistic, with control for the clinical center, was used for the analysis of categorical variables. The rate of survival free of target-vessel failure during the 270-day follow-up period was analyzed with the use of the

Table 1. Characteristics of the Patients and the Lesions.*

Characteristic	All Patients (N=1058)	Sirolimus-Stent Group (N=533)	Standard-Stent Group (N=525)
Age (yr)	62.3±11.1	62.1±11.2	62.4±11.0
Male sex (%)	71	73	70
Diabetes mellitus (%)	26	25	28
Hyperlipidemia (%)†	74	73	75
Hypertension (%)	68	68	68
Current smoker (%)	20	18	22
Previous myocardial infarction (%)	31	28	33
Angina pectoris (%)			
Exertional angina	58	59	59
Angina while at rest	23	23	23
Unstable angina‡	53	53	54
Target artery (%)			
Left anterior descending coronary artery	44	44	43
Right coronary artery	31	30	32
Left circumflex coronary artery	25	25	24
Multivessel disease (%)	42	41	42
ACC–AHA class (%)			
A	8	7	8
B1	36	34	38
B2	33	33	34
C	23	26	21
Diameter of reference vessel (mm)	2.80±0.47	2.79±0.45	2.81±0.49
Length of lesion (mm)	14.4±5.8	14.4±5.8	14.4±5.8

* Plus–minus values are means ±SD. There were no significant differences between the treatment groups. ACC denotes American College of Cardiology, and AHA American Heart Association.

† Hyperlipidemia was defined as a low-density lipoprotein cholesterol level above 130 mg per deciliter (3.4 mmol per liter).

‡ Unstable angina was defined according to the Braunwald classification.

actuarial life-table method, and the difference between survival curves was assessed with the log-rank test. To identify factors that might be related to angiographic restenosis and revascularization of the target lesion, logistic-regression models were used. All statistical analyses were performed with the use of SAS software (version 6.12, SAS Institute), and all reported P values are two-sided.

RESULTS

CHARACTERISTICS OF THE PATIENTS AND THE LESIONS

Between February 2001 and August 2001, 1101 patients gave written informed consent and were randomly assigned to one of the two treatment groups. After randomization, 43 patients (4 percent of all patients, 23 in the sirolimus-stent group and 20 in the standard-stent group) were withdrawn from the study and did not receive the assigned treatment. The reasons for withdrawal were the unavailability of the assigned type of stent at the center (in the cases of 4 patients) and the discovery of criteria for exclusion that became apparent only after pretreatment angiography (in 39 patients). The final patient cohort included 1058 patients — 533 in the sirolimus-stent group and 525 in the standard-stent group.

The groups were well matched, with no significant differences in the frequency of cardiac risk factors (Table 1). Among all patients, the mean age was 62 years; 71 percent were men, 31 percent had had a previous myocardial infarction, and 26 percent had diabetes. Cardiac symptoms included exertional angina in 58 percent of patients, angina while at rest in 23 percent, and unstable angina (Braunwald class I, II, or III) in 53 percent. The majority (56 percent) of treated lesions were class B2 or C according to the American College of Cardiology–American Heart Association classification, the average reference-vessel diameter was 2.80 mm, and the mean lesion length was 14.4 mm.

PROCEDURAL FACTORS

There were no differences between the groups in the rate of use of conventional interventions; glycoprotein IIb/IIIa inhibitors were given to 60 percent of patients, the maximal balloon-inflation pressure after stenting was 15 atm, and the mean (±SD) ratio of the stent length to the lesion length was 1.6±0.6. An average of 1.4 stents were implanted per target lesion, with overlapping stents in 28 percent of patients.

QUANTITATIVE CORONARY ANGIOGRAPHY

The dimensions of the lesion at base line were similar in the two groups (Table 2). Follow-up angiographic data were available for 350 patients in the sirolimus-stent group (86 percent of the patients assigned to undergo angiographic follow-up) and 353 in the standard-stent group (85 percent of the patients assigned to undergo angiographic follow-up). Table 2 shows that at follow-up, the minimal luminal diameter, stenosis as a percentage of the lu-

Table 2. Results of Quantitative Coronary Angiography.*

Variable	In-Stent Zone			In-Segment Zone		
	Sirolimus Stent	Standard Stent	P Value	Sirolimus Stent	Standard Stent	P Value
Minimal luminal diameter (mm)						
Before procedure	0.98±0.40	0.97±0.38	0.68	0.99±0.40	0.97±0.38	0.68
After procedure	2.67±0.40	2.68±0.42	0.98	2.38±0.45	2.40±0.46	0.63
At 240 days	2.50±0.58	1.69±0.79	<0.001	2.15±0.61	1.60±0.72	<0.001
Stenosis (% of luminal diameter)						
Before procedure	65.1±12.6	65.6±12.1	0.46	65.1±12.6	65.6±12.1	0.46
After procedure	5.4±8.2	6.0±7.9	0.22	16.1±9.7	16.2±8.5	0.80
At 240 days	10.4±16.5	40.1±25.3	<0.001	23.6±16.4	43.2±22.4	<0.001
Late luminal loss (mm)†	0.17±0.45	1.00±0.70	<0.001	0.24±0.47	0.81±0.67	<0.001
Restenosis (% of patients)‡	3.2	35.4	<0.001	8.9	36.3	<0.001

* Plus–minus values are means ±SD.

† Late luminal loss was defined as the difference between the minimal luminal diameter immediately after the placement of the stent and the minimal luminal diameter at 240 days. The data are for the 701 patients for whom both postprocedural and follow-up measurements of the minimal luminal diameter were available.

‡ Restenosis was defined as stenosis of 50 percent of the luminal diameter on the 240-day follow-up angiogram.

minal diameter, and the late luminal loss in both the in-stent zone and the in-segment zone were all improved with the sirolimus stent as compared with the standard stent (P<0.001 for all comparisons). The frequency of binary in-stent restenosis (stenosis of at least 50 percent of the luminal diameter) was 3.2 percent in the sirolimus-stent group and 35.4 percent in the standard-stent group (P<0.001), and the frequency of in-segment restenosis was 8.9 percent in the sirolimus-stent group and 36.3 percent in the standard-stent group (P<0.001). The higher rate of in-segment restenosis in the sirolimus-stent group was due to a smaller reduction in late luminal loss in the in-segment zone than in the in-stent zone and a higher rate of restenosis at the proximal margin of the stent than at the distal margin or in the body of the stent.

INTRAVASCULAR ULTRASONOGRAPHY

The use of sirolimus-eluting stents, as compared with the use of standard stents, resulted in reductions in the neointimal volume in the in-stent zone (4.4 mm^3 vs. 57.6 mm^3, P<0.001) and in the in-stent obstruction as a percentage of volume (3.1 percent vs. 33.4 percent, P<0.001).

CLINICAL OUTCOMES

Major adverse cardiac events are listed in Table 3. In-hospital events occurred at a similar frequency in the two groups (including death, myocardial infarction, and repeated revascularization); the proportion of patients with any in-hospital major adverse event was 2.4 percent in the sirolimus-stent group and 1.5 percent in the standard-stent group (P=0.38). There was a lower rate of out-of-hospital adverse events during the 270 days of follow-up in the sirolimus-stent group than in the standard-stent group; reductions included those in the number of patients with non–Q-wave myocardial infarction (from 7 to 1, P=0.04), the number requiring revascularization of the target lesion (from 87 to 21, P<0.001), and the number with any major adverse event (from 93 to 26, P<0.001). Similarly, the number of patients reaching the primary clinical end point, failure of the target vessel within 270 days, was reduced by 58 percent with sirolimus stents (from 110 to 46, P<0.001). The rate of survival free of target-vessel failure for 270 days increased from 78.6 percent with a standard stent to 91.1 percent with a sirolimus stent (P<0.001) (Fig. 1).

Stent thrombosis was infrequent, and the rate was similar in the two treatment groups. There were no acute stent thromboses (occurring less than 24 hours after placement of the stent), there was one case of subacute stent thrombosis (occurring between 1 and 30 days after placement) in each group, and there were four late stent thromboses (occurring between 31 and 270 days after placement) — one in the sirolimus-stent group and three in the standard-stent group. The cumulative frequency of stent

Table 3. Major Adverse Cardiac Events in the Hospital and outside of the Hospital during 270 Days of Follow-up.*

Variable	Sirolimus-Stent Group (N=533)	Standard-Stent Group (N=525)	P Value
	no. of patients (%)		
In-hospital events			
Death	1 (0.2)	0	
Myocardial infarction	12 (2.3)	8 (1.5)	
Q-wave	2 (0.4)	0	
Non–Q-wave	10 (1.9)	8 (1.5)	
Target-lesion revascularization	1 (0.2)	0	
CABG	0	0	
PTCA	1 (0.2)	0	
Any major adverse cardiac event	13 (2.4)	8 (1.5)	
Out-of-hospital events			
Death	4 (0.8)	3 (0.6)	
Myocardial infarction	3 (0.6)	9 (1.7)	
Q-wave	2 (0.4)	2 (0.4)	
Non–Q-wave	1 (0.2)	7 (1.3)	0.04
Target-lesion revascularization	21 (3.9)	87 (16.6)	<0.001
CABG	3 (0.6)	8 (1.5)	
PTCA	19 (3.6)	83 (15.8)	<0.001
Any major adverse cardiac event	26 (4.9)	93 (17.7)	<0.001
Cumulative to 270 days			
Death	5 (0.9)	3 (0.6)	
Myocardial infarction	15 (2.8)	17 (3.2)	
Q-wave	4 (0.8)	2 (0.4)	
Non–Q-wave	11 (2.1)	15 (2.9)	
Target-lesion revascularization	22 (4.1)	87 (16.6)	<0.001
CABG	3 (0.6)	8 (1.5)	
PTCA	20 (3.8)	83 (15.8)	<0.001
Any major adverse cardiac event	38 (7.1)	99 (18.9)	<0.001
Target-vessel failure	46 (8.6)	110 (21.0)	<0.001
Stent thrombosis	2 (0.4)	4 (0.8)	

* P values are given only for significant differences. The total numbers of patients who underwent target-lesion revascularization may not equal the number who underwent coronary-artery bypass grafting (CABG) plus the number who underwent percutaneous transluminal coronary angioplasty (PTCA), because some patients underwent both procedures; the numbers given for any major adverse cardiac event in the cumulative analysis do not equal the numbers given for in-hospital events plus out-of-hospital events, because some patients had more than one event.

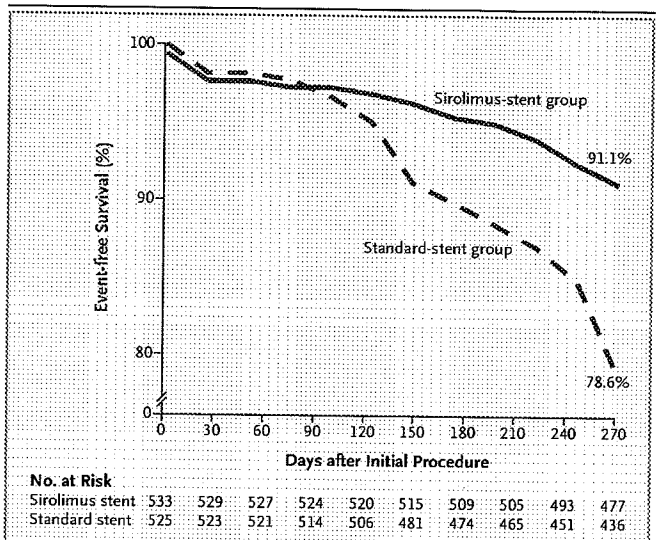

Figure 1. Actuarial Rate of Survival Free from Target-Vessel Failure among Patients Who Received Either a Sirolimus-Eluting Stent or a Standard Stent.

The rate of event-free survival was significantly higher in the sirolimus-stent group than in the standard-stent group (P<0.001 by the Wilcoxon and log-rank tests).

thrombosis was 0.4 percent in the sirolimus-stent group and 0.8 percent in the standard-stent group.

SUBGROUP ANALYSES, MULTIVARIABLE ANALYSES, AND ASSESSMENTS OF TREATMENT EFFECTS

Among the 279 patients with diabetes (26 percent of the total study population; 131 patients in the sirolimus-stent group and 148 in the standard-stent group), the absolute frequency of in-segment restenosis and the absolute frequency of target-lesion revascularization were higher than those among patients without diabetes in both treatment groups, but the relative reductions after the placement of a sirolimus stent were of similar magnitude (the rate of in-segment restenosis was reduced from 50.5 percent to 17.6 percent, P<0.001; and the rate of target-lesion revascularization was reduced from 22.3 percent to 6.9 percent, P<0.001).

Among the third of the patient population with the smallest vessels (averaging 2.32 mm in diameter in the sirolimus-stent group and 2.29 mm in the standard-stent group), there was less (albeit still significant) improvement with sirolimus stents in both the rate of in-segment restenosis (18.4 percent, vs. 42.9 percent in the standard-stent group; P<0.001) and the rate of target-lesion revascularization (7.3 percent vs. 20.6 percent, P<0.001). Among the patients with the smallest vessels who received sirolimus stents, the restenosis was usually located at the proximal margin of the stent.

In addition to reducing the overall frequency of angiographic restenosis, the use of sirolimus stents altered the patterns of post-stenting restenosis. The mean length of a restenotic lesion was 9.1±5.8 mm after the placement of a sirolimus stent, as compared with 14.8±7.4 mm after the placement of a standard stent (P<0.001), with a diffuse pattern (a lesion length of more than 10 mm) in 58 percent of cases after the placement of a standard stent, as compared with only 13 percent of cases after the placement of a sirolimus stent (P<0.001).

The association of known risk factors for restenosis with the treatment effect of the sirolimus stent on either angiographic or clinical restenosis was evaluated with the use of multivariable logistic-regression modeling of the rate of in-segment restenosis within 240 days and the rate of target-lesion revascularization within 270 days. In the model of in-segment restenosis, diabetes was significantly associated with an increased risk of restenosis (odds ratio, 2.39; P<0.001), as were the diameter of the reference vessel (odds ratio per 1-mm decrement, 0.54; P=0.001) and the length of the lesion (odds ratio per 1-mm increment, 1.02; P=0.01).

Similarly, in the model of target-lesion revascularization, diabetes was significantly associated with an increased risk of restenosis (odds ratio, 1.65; P=0.03), as were the diameter of the reference vessel (odds ratio per 1-mm decrement, 0.37; P<0.001) and the length of the lesion (odds ratio per 1-mm increment, 1.05; P<0.001). According to both of these models, assignment to the sirolimus-stent group was associated with a significant reduction in the risk of restenosis (odds ratio for in-segment restenosis, 0.24; odds ratio for target-lesion revascularization, 0.17; P<0.001 for both comparisons).

Figure 2 shows the consistent beneficial effect of sirolimus-eluting stents on the risk of target-lesion revascularization in important clinical and angiographic subgroups, including those defined according to sex, the presence or absence of diabetes, whether or not the lesion was located in the left anterior descending artery, the size of the vessel, the length of the lesion, and the presence or absence of overlapping stents.

Variable	No. of Patients	Rate of Target-Lesion Revascularization (%)	
		Sirolimus stent	Standard stent
Overall	1058	4.1	16.6
Male sex	753	4.4	16.6
Female sex	305	3.4	16.5
Diabetes			
Yes	279	6.9	22.3
No	779	3.2	14.3
Left anterior descending coronary artery			
Yes	462	5.1	19.8
No	596	3.4	14.3
Vessel diameter			
Small <2.75 mm	522	6.3	18.7
Large ≥2.75 mm	523	1.9	14.8
Lesion length			
≤13.5 mm	519	3.2	16.1
>13.5 mm	519	5.2	17.4
Overlapping stents			
Yes	344	4.5	17.7
No	714	3.9	16.1

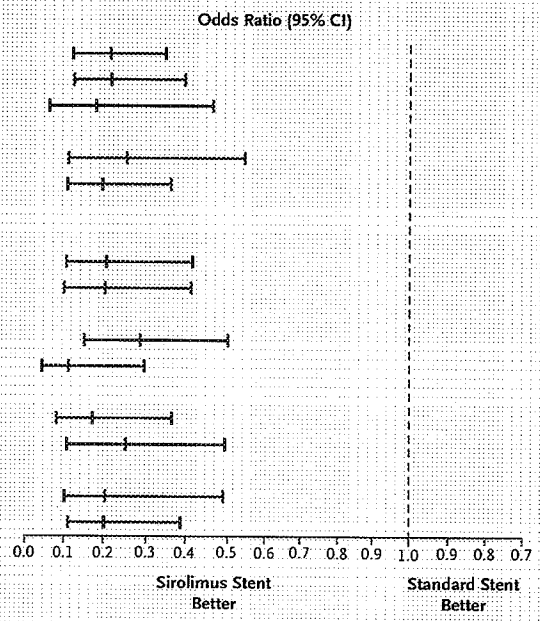

Figure 2. Rates of Target-Lesion Revascularization (Either Percutaneous Transluminal Coronary Angiography or Coronary-Artery Bypass Grafting) and Odds Ratios at 270 Days for Various Subgroups of Patients.

For the analyses in terms of vessel diameter and lesion length, the variable was dichotomized at the median value. P<0.001 for all comparisons between groups. CI denotes confidence interval.

DISCUSSION

In comparison with previous studies of sirolimus-eluting stents,[23-25] our trial enrolled patients with more challenging conditions, including a higher frequency of cardiac risk factors (especially diabetes), more complex lesion morphology, and longer lesions. Nevertheless, the suppression of in-stent neointimal hyperplasia was again demonstrated after the placement of sirolimus-eluting stents, both on angiography (an 83 percent reduction in late luminal loss and a 91 percent reduction in the rate of in-stent restenosis) and on intravascular ultrasonography (a 92 percent reduction in neointimal volume). Moreover, the clinical manifestations of excessive neointimal hyperplasia were similarly improved, with a 77 percent reduction in the rate of out-of-hospital target-lesion revascularization and an 85 percent reduction in the rate of out-of-hospital non–Q-wave myocardial infarction. There were no untoward angiographic complications (e.g., late aneurysms), and the rates of adverse clinical events (including stent thromboses) were not significantly higher in the sirolimus-stent group than in the standard-stent group.

The subgroup analyses indicated that after the placement of a sirolimus stent, the exposed margins of stents that did not cover the entire region of balloon injury were the primary sites of restenosis, which occurred predominantly at the proximal stent margin in smaller vessels. Thus, we would recommend the use of a technique including predilation with shorter balloons, the use of longer single stents in order to cover the entire zone of balloon injury, and dilation after stenting (as needed) with short, high-pressure balloons within the stented regions.

In addition to the reduction in the frequency of restenosis, the pattern of post-stenting restenosis

differed with sirolimus-eluting stents: whereas restenotic lesions in standard stents were diffuse, those in sirolimus-eluting stents were focal.[26] Such focal post-stenting lesions may typically be treated successfully with the use of simple balloon angioplasty,[27,28] minimizing the need for subsequent vascular brachytherapy.[29,30] Both patients with diabetes and those with lesions in smaller vessels have higher absolute rates of restenosis, although the relative reduction in the rate of restenosis is preserved. Most important, the sirolimus-eluting stent was found to have a consistent treatment effect in analyses of a broad range of subgroups of patients and lesions.

To determine the ultimate clinical usefulness of sirolimus-eluting stents, additional clinical trials are required that involve patients with disease in a bifurcation, chronic total occlusions, saphenous-vein graft disease, restenosis after stenting, failure of vascular brachytherapy, lesions in the left main coronary artery, and multivessel disease. The findings in two-year follow-up examinations in a cohort of 45 patients who were treated with sirolimus-eluting stents are encouraging, indicating that the angiographic and clinical efficacy are maintained.[31] However, the long-term safety and durability of this very potent site-specific therapy require further substantiation in larger cohorts of patients.

A clinically efficacious drug-eluting stent system requires a meticulous integration of the stent design, drug-carrier vehicle, and therapeutic agent. Preliminary stent-based results with paclitaxel, a well-described chemotherapeutic agent that suppresses microtubule dynamics,[32-34] delivered through a polymer-matrix formulation, have also shown promise.[35] The results of our clinical trial demonstrate that the sirolimus-eluting stent has achieved the delicate balance of preserved safety and improved efficacy and thus has the potential to alter the course of coronary therapy in the future.

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Dr. Moses reports having received consulting fees from Cordis (Johnson & Johnson) and lecture fees from Johnson & Johnson and holding equity interests in Johnson & Johnson; Dr. Leon reports having received consulting and lecture fees from and holding equity interests in Cordis; Dr. Popma reports having received consulting fees, lecture fees, and grant support from Cordis; Dr. Fitzgerald reports having received consulting fees, lecture fees, and grant support from Cordis; Dr. Kereiakes reports having received honorariums and research grants from Johnson & Johnson (Centocor); Dr. Williams reports having received consulting fees and grant support from Cordis; and Dr. Teirstein reports having received consulting and lecture fees from and holding equity interests in Cordis, and having received royalties from Cordis related to the sale of radiation-delivery catheters.

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APPENDIX

The following investigators and institutions participated in the multicenter, randomized, double-blind study of the sirolimus-eluting balloon-expandable stent in the treatment of patients with de novo native coronary-artery lesions (the SIRIUS trial): Sponsor — Cordis, Warren, N.J., D. Donohoe (medical director), J. Jaeger (program director), E. Keim, L. Lonzetta, L. Reynolds, J. Batiller, C. Hill; Data and Safety Monitoring Board — B. Gersch (chair), Rochester, Minn.; M. Farkouh, New York; R. Bonow, Chicago; R. D'Agostino (biostatistician), Boston; G. Mintz, Washington, D.C.; A. Schwartz, New York; Data Management — Harvard Clinical Research Institute, Boston; *Coordination* — E. Catapane; *Clinical Events Committee* — D. Cohen (chair), L. Epstein, J. Kannam, W. Manning, J. Markis; Electrocardiography Core Laboratory — P. Zimetbaum, M. Josephson; Core Angiographic Laboratory — Brigham and Women's Hospital, Boston, J. Popma (director); Core Intravascular Ultrasound Laboratory — Stanford University Medical Center, Stanford, Calif., P. Fitzgerald (director); Clinical Sites — J. Carrozza, P. Rooney, Beth Israel Deaconess Medical Center, Boston; S. Ellis, A. Robakowski, Cleveland Clinic Foundation, Cleveland; J. Douglas, P. Hyde, Emory University Hospital, Atlanta; J. Moses, M. Leon, V. Laroche, Lenox Hill Hospital, New York; P. Teirstein, E. Anderson, Scripps Clinic, La Jolla, Calif.; E. Perin, M. Harlan, Texas Heart Institute, Houston; R. Wilensky, M. Walsh, Hospital of the University of Pennsylvania, Philadelphia; L. Satler, J. Lavoie, Washington Hospital Center, Washington, D.C.; M. Cleman, C. Roberts, Yale University Hospital, New Haven, Conn.; S. DeMaio, L. Rogers, Baylor Medical Center, Dallas; E. Fry, A. Taylor, M. Potrikus, Saint Vincent's Hospital, Indianapolis; A. Yeung, C. McWard, Stanford University Medical Center, Stanford, Calif.; J. Zidar, S. Dickerson, Duke University Medical Center, Durham, N.C.; W. O'Neill, K. Dimick, William Beaumont Hospital, Royal Oak, Mich.; G. Mishkel, J. Daniels, P. Sullivan, Saint John's Hospital, Springfield, Ill.; D. McCormick, L. Mark, B. Connor, Hahnemann Hospital, Philadelphia; D. Roberts, B. Seiler, Sutter Memorial General Hospital, Sacramento, Calif.; D. Holmes, D. Shelstad, Saint Mary's Hospital, Rochester, Minn.; F. Kiernan, D. Murphy, Hartford Hospital, Hartford, Conn.; M. Midei, E. Yaker, Saint Joseph's Hospital, Baltimore; D. Williams, J. Muratori, T. Chaffee, Rhode Island Hospital, Providence; T. Fischell, S. Baskerville, Borgess Medical Center, Kalamazoo, Mich.; S. Oesterle, I. Palacios, C. Cothern, Massachusetts General Hospital, Boston; S. Yakubov, C. Gilliland, P. Vieira, Riverside Methodist Hospital, Columbus, Ohio; D. Kereiakes, R. Lengerich, Christ Hospital–Lindner Center, Cincinnati; C. Davidson, L. Eckman, Northwestern Memorial Hospital, Chicago; C. Brown, K. Reid, Piedmont Hospital, Atlanta; C. Lambert, T. Watts, N. Parker, Health First Institute, Melbourne, Fla.; D. Baim, R. Monboquette, Brigham and Women's Hospital, Boston; A. Raizner, R. Benfield, Methodist Hospital, Houston; B. Cohen, R. Lao, Morristown Memorial Hospital, Morristown, N.J.; N. Laufer, M. Balfour, Good Samaritan Regional Medical Center, Phoenix, Ariz.; S. Raible, B.J. Henehan, Jewish Hospital Heart and Lung Institute, Louisville, Ky.; P. Coleman, A. Nofi, Northern California Medical Association, Santa Rosa; S. Sorenson, K. Robinson, Latter Day Saints Hospital, Salt Lake City; M. Mooney, P. Demmer, Abbott Northwestern Hospital, Minneapolis; T. Feldman, J. Lopez, L. Loftis, University of Chicago Hospitals, Chicago; J. Lasala, K. Zuchowski, S. Aubuchon, Barnes Jewish Hospital, St. Louis; R. Caputo, C. Lastinger, Saint Joseph's Hospital, Syracuse, N.Y.; C. O'Shaughnessy, T. Julio, L. St. Marie, L. Barr, North Ohio Heart Center, Elyria; H. Madyoon, T. Weaver, Saint Joseph's Medical Center, Stockton, Calif.; J. Midwall, L. Herlan, JFK Memorial Hospital, Atlantis, Fla.; M. Bates, L. Lukhart, Charleston Area Medical Center, Charleston, W.Va.; M. Clark, L. Pennington, Integris Oklahoma Heart Institute, Okla-

homa City; T. Vellinga, K. McCormick, S. Congemi, Saint Luke's Medical Center, Milwaukee; C. Simonton, C. Dellinger, G. Schwartz, Sanger Clinic, Charlotte, N.C.; F. Leya, D. Jednachowski, Loyola University Medical Center, Maywood, Ill.; G. Chapman, D. Gargus, University of Alabama, Birmingham; M. Reisman, F. Clouarte, Swedish Heart Hospital, Seattle; S. Wong, D. Reynolds, Cornell University and New York Presbyterian Hospital, New York; T. Bass, G. Morris, V. Brooks, University of Florida Health Science Center, Jacksonville; B. Bachinsky, A. Todd, C. Schaeffer, R. Yost, Harrisburg Hospital, Wormleysburg, Pa.; M. Buchbinder, J. Logan, Foundation for Cardiovascular Medicine, La Jolla, Calif.

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Inter Partes Reexamination No. 95/001,095
Declaration of Antonios G. Mikos, Ph.D.
Exhibit 10

Percutaneous Polymeric Stents in Porcine Coronary Arteries

Initial Experience With Polyethylene Terephthalate Stents

Joseph G. Murphy, MD; Robert S. Schwartz, MD; William D. Edwards, MD;
Allan R. Camrud, RN; Ronald E. Vlietstra, MB,ChB; and David R. Holmes Jr., MD

Background. **To test the feasibility of percutaneous deployment of intracoronary polymeric stents, a prototype polyethylene terephthalate (PET) stent and a catheter-based delivery system were developed.**

Methods and Results. **Polymeric stents were deployed in the coronary arteries of 11 Yucatan swine: six stents were placed in the left anterior descending coronary artery, four stents were placed in the circumflex artery, and one stent was placed in the right coronary artery. Stent deployment was achieved by withdrawal of an outer delivery sheath, thus allowing the PET stent to self-expand to a preformed configuration. Two animals died during surgery, one during stent placement and the other several hours after implantation due to intracoronary thrombus formation. Two animals were electively sacrificed within 24 hours of stent implant to examine the adequacy of stent deployment within the coronary vessel. The remaining seven animals survived until the termination of the study 4–6 weeks later. Light microscopic examination of the stented vessels showed an extensive neointimal proliferative response with vessel occlusion in all animals who survived initial stent placement. There were two distinct types of histological responses to the PET stent—a chronic foreign body inflammatory response around the stent tines and a neointimal proliferative response in the center of the occluded vessel lumen. The histological response seen in the central area of the vessel was morphologically similar to that seen in patients with restenosis after successful percutaneous transluminal coronary angioplasty, whereas the morphological response seen at the periphery of the stent tine was similar to that exhibited by a chronic foreign body reaction and was not typical of that seen in a restenosis lesion. A ventricular aneurysm also developed in the area of myocardium that was previously supplied by the occluded vessel.**

Conclusions. **This study demonstrates that percutaneous deployment of polymeric stents in the coronary arteries is technically feasible. The use of PET polymer was associated with an intense proliferative neointimal response that resulted in complete vessel occlusion. Histological examination of the stented segments of the vessel revealed no evidence that dissection of the vessel wall had occurred at the time of initial stent deployment. Although the PET polymer was of similar quality to that used in the manufacture of balloon angioplasty catheters, a toxic chemical or contaminant effect cannot be completely excluded as the stimulus to intimal proliferation. This finding may have relevance to the selection of materials for use as intravascular devices. (*Circulation* 1992;86:1596–1604)**

KEY WORDS • **stent** • **coronary restenosis** • **ventricular aneurysm**

Percutaneous implantation of metallic stents in the coronary vessels was first reported in humans in 1987 by Sigwart et al.[1] Despite the high initial success rate, early and late complications have been reported with all current metallic stent designs.[2–5] These problems include acute stent occlusion due to thrombus formation and restenosis due to intimal proliferation through the stent meshwork. Compared with stainless-steel or tantalum stents, polymeric stents have the potential to be biodegradable and/or drug eluting.

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To test the hypothesis that percutaneous placement of polymeric stents was technically feasible, we developed a polyethylene terephthalate (PET) intracoronary stent and a specially adapted catheter-based delivery system. We report the successful deployment of percutaneous intracoronary polymeric stents in 11 Yucatan swine. Seven animals survived until the termination of the study 4–6 weeks after stent implant.

Methods

Animals

All studies were conducted with the approval of and adherence to the ethical guidelines of the Mayo Clinic Animal Care and Use Committee.

The histological features of swine coronary arteries are similar to those of human coronary arteries, and the development of spontaneous atherosclerotic lesions is well documented in the pig.[6,7]

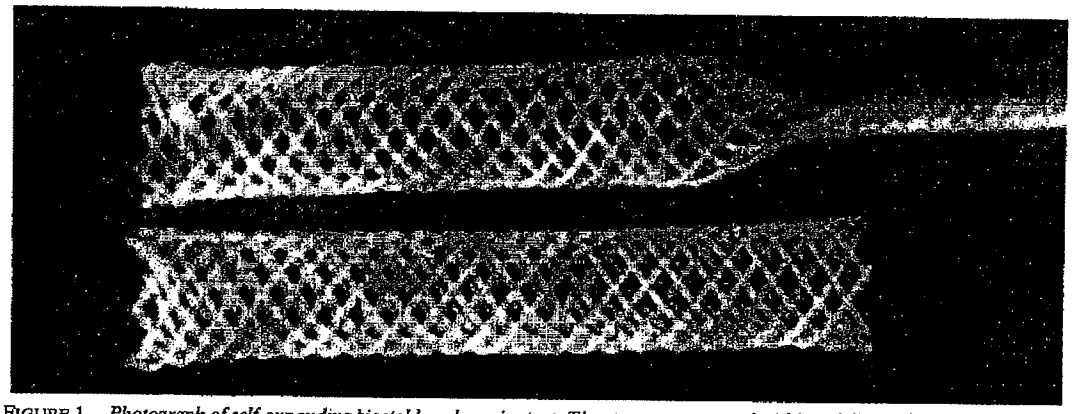


FIGURE 1. *Photograph of self-expanding biostable polymeric stent. The stent was mounted within a delivery sheath (top) that was withdrawn. After self-expansion, the delivery catheter was removed, leaving the expanded meshwork stent (bottom) in place.*

Pigs (weight, 40–50 kg) were fed a standard natural grain diet without lipid or cholesterol supplementation throughout the study.

Procedure

The carotid artery was used for arterial access in all animals. A midline incision was made in the ventral area of the neck after infiltration with 1% xylocaine. General anesthesia was induced with intramuscular ketamine (12 mg/kg) and xylazine (8 mg/kg). Continuous ECG monitoring and ear oximetry were performed throughout the procedure. The right external carotid artery was surgically exposed, and an 8F intra-arterial sheath was placed over a 0.035-in. tight J wire. Heparin (400 USP units/kg) was administered intravenously as a bolus. The carotid approach was chosen over the femoral approach because of the lower occurrence of postoperative hematoma formation, which is common after administration of a large dose of heparin following a femoral arteriotomy in swine.

The coronary artery was engaged using standard techniques with an 8F angioplasty guide catheter under fluoroscopic visualization. The unconstrained stent diameter was 2.5 mm, and the length was 2.0 cm. The delivery sheath was made from commercially available polypropylene tubing with an internal diameter of 1.4 mm and wall thickness of 0.3 mm to give an outside diameter of 2.0 mm. The filament mesh was 12 filaments per centimeter length, and the individual filaments were approximately 0.12 mm in diameter. The expandability ratio (ratio of expanded diameter to collapsed diameter) was 1.79.

The polymeric stent was loaded into the specially designed delivery catheter just before deployment. This was done to avoid stent distortion due to polymeric cold flow, which occurs when a polymeric stent is maintained in a contracted configuration for an extended period of time. The stent deployment catheter was advanced into the left anterior descending coronary, circumflex, or right coronary artery, as per protocol. The stents remained in their contracted configuration within an outer delivery sheath until the time of deployment (Figure 1). All stents were deployed in the main vessel trunk away from branch points or areas where the vessel tapered rapidly. For stent deployment, the outer delivery sheath was withdrawn so that the stent could self-expand within the coronary vessel (Figure 2).

Injection of contrast medium 15 minutes after the procedure confirmed acute vessel patency in all animals without evidence of filling defects, vessel wall dissection, or distal coronary spasm. The carotid arteriotomy was repaired using standard technique or ligated if repair was not feasible, and the neck incision was closed with interrupted sutures. The animals were returned to a postoperative recovery area and observed. No antiplatelet agent or additional anticoagulants were administered subsequent to the initial bolus of heparin.

Histopathology

Seven pigs were sacrificed with intravenous injections of barbiturate and potassium chloride 4–6 weeks after stent implantation. Hearts were pressure-perfused for 24 hours with 10% neutral buffered formalin. Segments of coronary arteries that contained the polymeric stents were easily identified by transilluminating the entire vessel after removal from the heart using a powerful light source. In addition, it was frequently possible to palpate the polymeric stent tines within the coronary vessel at the time of histological dissection. These segments were removed from the heart, with at least 1 cm of normal vessel proximal and distal to the stent. The stent filaments were removed (so as not to distort or damage the artery) after the vessel was cross sectioned at 2–3-mm intervals. Sections from each arterial segment were stained with hematoxylin and eosin and with Lawson's elastic-van Gieson stains.

Results

Eleven animals underwent successful polymeric stent implantation (Table 1). All had patent vessels (determined by angiography) 15 minutes after stent implant (Figure 3). Seven animals survived until the termination of the study 4–6 weeks after implantation. Two animals died during surgery – one due to vessel occlusion secondary to an intimal dissection caused by trauma from the stent delivery catheter and the other several hours after stent implantation due to thrombus formation in the stent. There was no intimal dissection, and the stent was correctly deployed. Two animals were sacrificed within 24

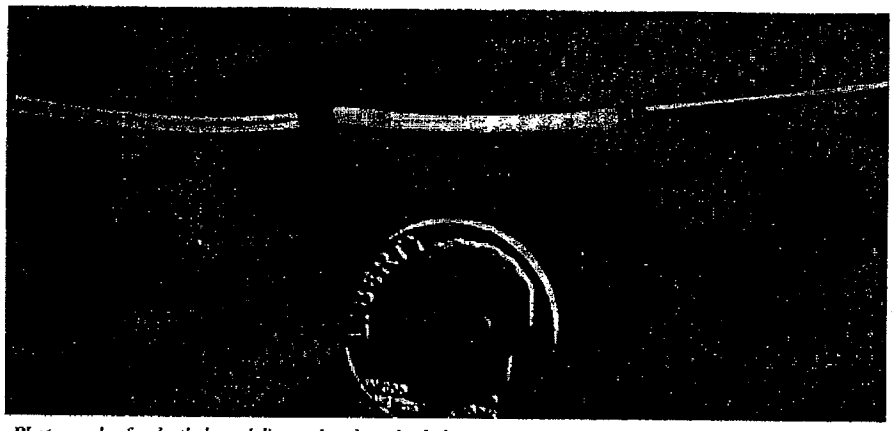


FIGURE 2. *Photograph of polyethylene delivery sheath, polyethylene terephthalate stent in contracted configuration, and metallic expulsion rod as seen from left to right. The expulsion rod was used to expel the stent as the delivery sheath was withdrawn.*

hours after stent implant to confirm the correct mechanical deployment of the stent within the coronary vessel.

All animals that were sacrificed 4–6 weeks after stent implant had vessel occlusion at the site of stent deployment (Figure 4). Light microscopy showed that vessel occlusion was due to neointimal proliferation at the site of stent implantation (Figure 5A). Two histological patterns were seen: a chronic foreign body inflammatory response with lymphocytes, eosinophils, and giant cells surrounding the stent filaments (Figure 5B) and a marked neointimal proliferative response (Figure 6A) in the center of the vessel. The neointimal proliferative response was morphologically similar to that seen in human restenotic tissue obtained at the time of directional atherectomy or autopsy. The neointimal proliferation in the center of the vessel was histologically distinct from the chronic inflammation and was of smooth muscle cell origin, as established by positive staining for actin (Figure 6B). Electron microscopy also confirmed the presence of contractile elements (a feature of smooth muscle cells and myofibroblasts) in these cells. Vessel occlusion by proliferative neointima occurred in all seven animals.

An unexpected finding was the development of postinfarction ventricular aneurysms in all animals sacrificed 4–6 weeks after stent implant (Figure 7A). A cross section of the aneurysmal wall showed replacement of ventricular muscle by scar tissue (Figure 7B). The pig has not previously been recognized as an experimental model for post–myocardial infarct ventricular aneurysm formation.

Discussion

This study demonstrated the technical feasibility, at least in the short term, of implantation of polymeric stents in coronary vessels. Complete vessel occlusion by neointima occurred in all animals surviving to elective sacrifice. Ventricular aneurysm formation was an unexpected late finding probably induced by the PET material of which the stent was composed. Other polymers may not incite a similar reaction.

Acute stent occlusion due to thrombus formation has been a significant limiting factor in the development of intravascular metallic stents. In an early study with the metallic Wallsten stent, there was partial or complete stent occlusion by thrombus in 13 of 43 animals.[3] In our study, no antiplatelet agents and no anticoagulants were administered with the exception of a single periprocedural bolus of heparin. In all animals (with one exception), there was no evidence of acute occlusive intravascular thrombus formation.

An important point in this study is whether insufficient expansile force was applied to the polymeric stent that would thus render the device in an intraluminal position and therefore an obstructive and highly thrombogenic position. There were three reasons for believing that this was not so. First, angiography performed 15 minutes after stent implant showed the vessel to be widely patent without evidence of an obstructive intraluminal thrombus. The polymeric stent material itself was not visible by radiographic means, but there was no indirect evidence to suggest thrombus formation within the device. Second, it has been our experience, when using metallic stents in porcine coronary arteries, that

TABLE 1. Vessel Occlusion and Ventricular Aneurysm After Polymeric Stent Implantation in Pigs

Animal	Artery	Survival after operation (days)	Vessel occlusion at death	Formation of ventricular aneurysm
1	LAD	39	+	+
2	LAD	40	+	+
3	LAD	41	+	+
4*	LAD	0	–	–
5	LCx	42	+	+
6	LAD	39	+	+
7†	LAD	0	+	–
8	LCx	29	+	+
9	LCx	28	+	+
10*	RCA	0	–	–
11‡	LCx	0	+	–

LAD, left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.
*Elective sacrifice within the first 24 hours after stent implant.
†Died of an intracoronary thrombus several hours after stent implant.
‡Died during stent placement.

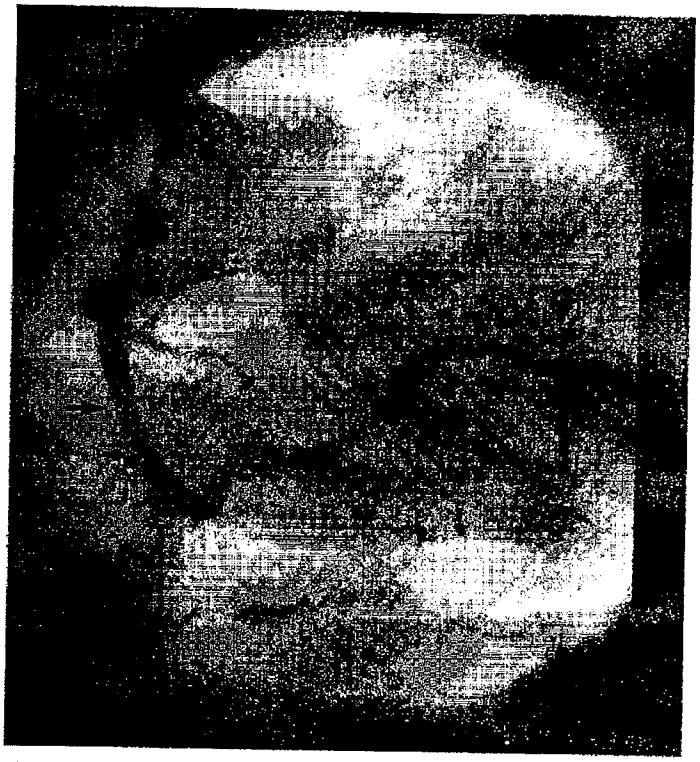


FIGURE 3. *Coronary angiogram of the right coronary artery after deployment of a polyethylene terephthalate polymeric stent. Arrow indicates the site of the stent placement. By the nature of their biomaterials, polymeric stents are not directly visible on angiography. This angiogram shows normal vessel patency at the site of deployment and the absence of angiographically visible thrombus.*

incomplete deployment of the stent that resulted in the formation of an acute obstructive thrombus always caused animal death due to ventricular fibrillation. This finding has also been noted by other investigators and probably results from the absence of any significant collateral circulation in the porcine model. Thus, it can be inferred that if animal survival occurred in the short term, an acute obstructive thrombus was almost certainly not present. Third, the position of the stent tines can be seen on the final histological sections of all vessels. In these sections, the holes where the stent tines had been placed can be seen to be subadjacent to the

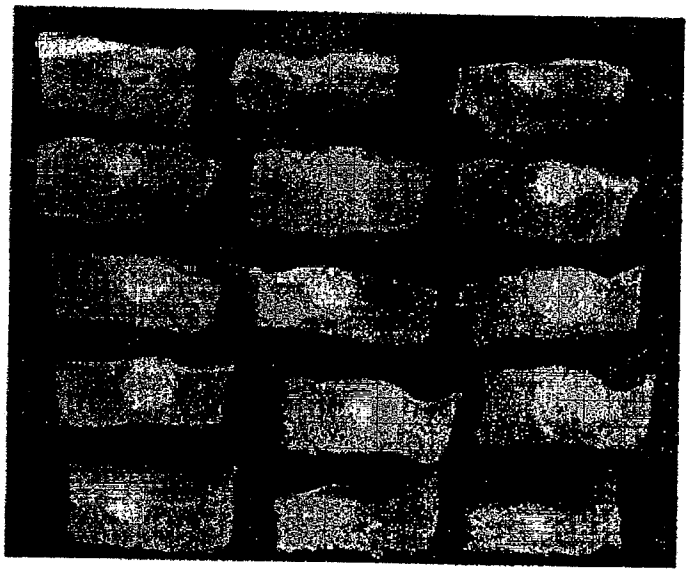
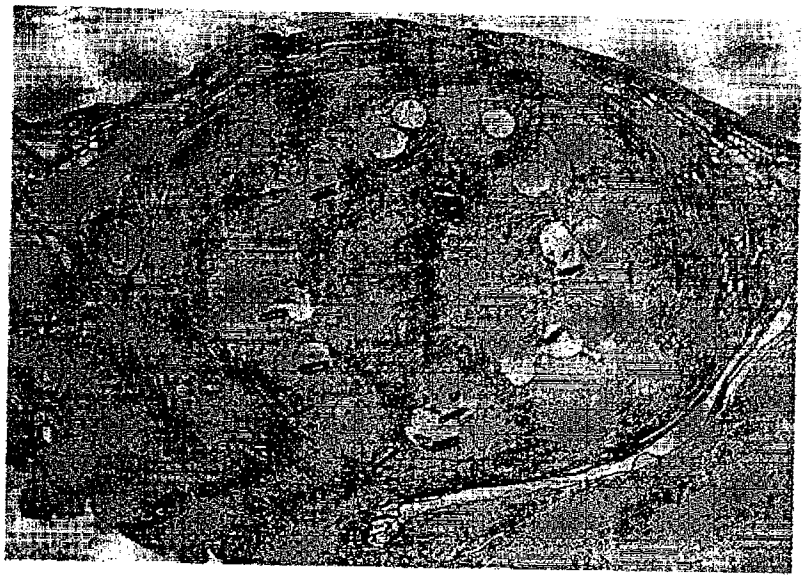


FIGURE 4. *Photographs of luminal occlusion resulting from polymeric stent placement. These cross sections were taken from the same left anterior descending coronary artery at 2–3-mm intervals. The upper-left section was proximal to the site of the stent. Abnormal vessel segments with marked proliferation and vessel occlusion are visible where the stent filaments had been deployed.*



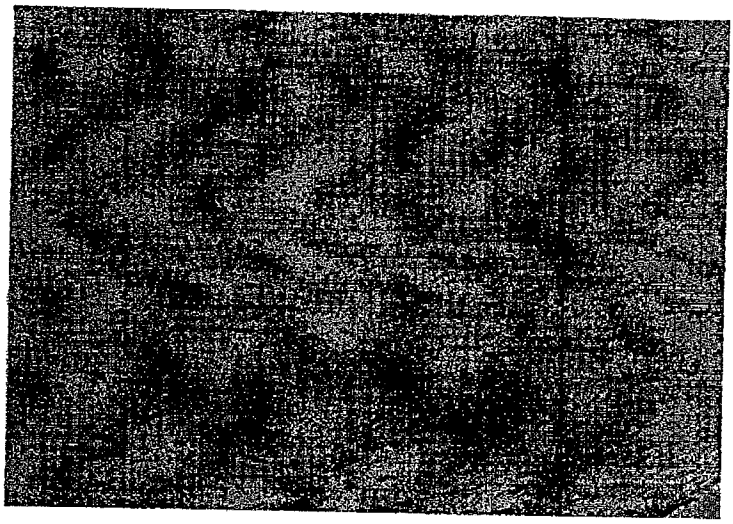
A

B

FIGURE 5. Panel A: *Photomicrograph of marked neointimal proliferation and vessel occlusion. There is histological evidence of limited damage to the arterial wall. The medial and adventitial architecture is intact, but there is disruption of the internal elastic lamina at the 5- and 10-o'clock positions. (van Gieson stain; original magnification, ×24.)* Panel B: *Photomicrograph of marked chronic foreign body inflammatory response in a circumferential pattern around the stent wires* (arrow). *(Hematoxylin and eosin stain; original magnification, ×119.)*

vessel wall. It is clear from these histological sections that complete stent expansion occurred in all animals.

Thus, in summary, the final histological pattern of the stent tines as seen at the time of being sacrificed, the fact that the animals survived to elective death, and the angiographic pattern immediately after stent implantation strongly support the argument that adequate stent expansion did occur and that the stent had not been improperly or incompletely expanded (which would have rendered it obstructive and highly thrombogenic). Equally, there was no evidence that overexpansion of the stent occurred at the time of implant. First, the radial expansive force of a polymeric stent is low and would probably not be capable of resulting in a medial



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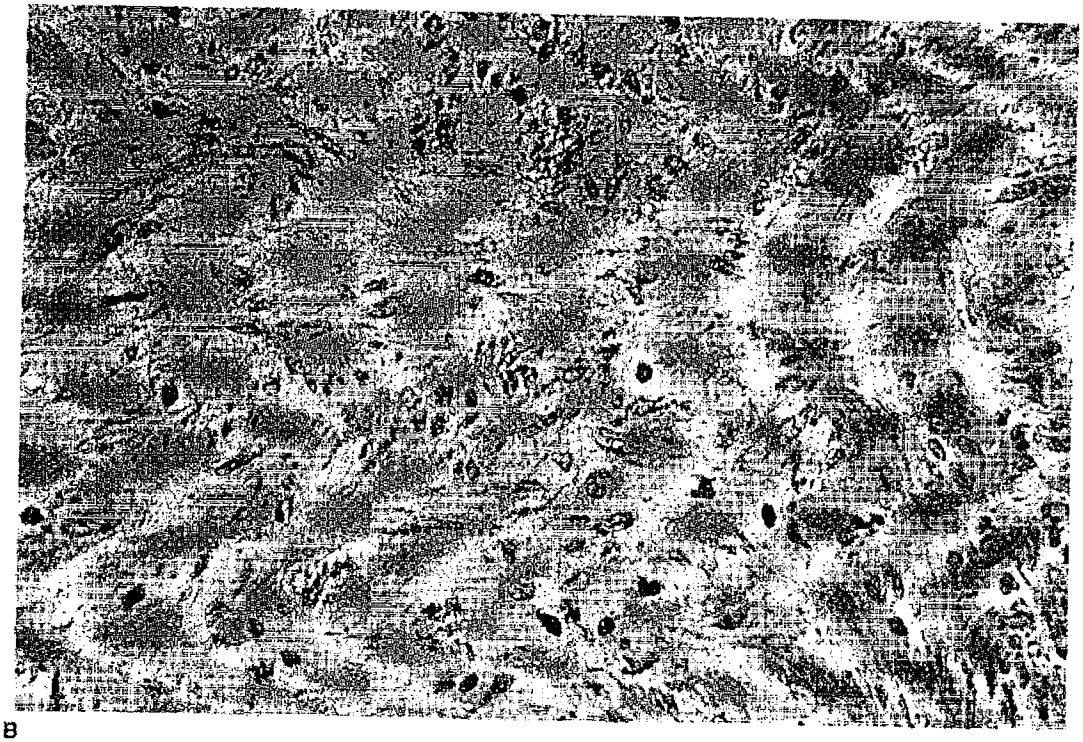


B

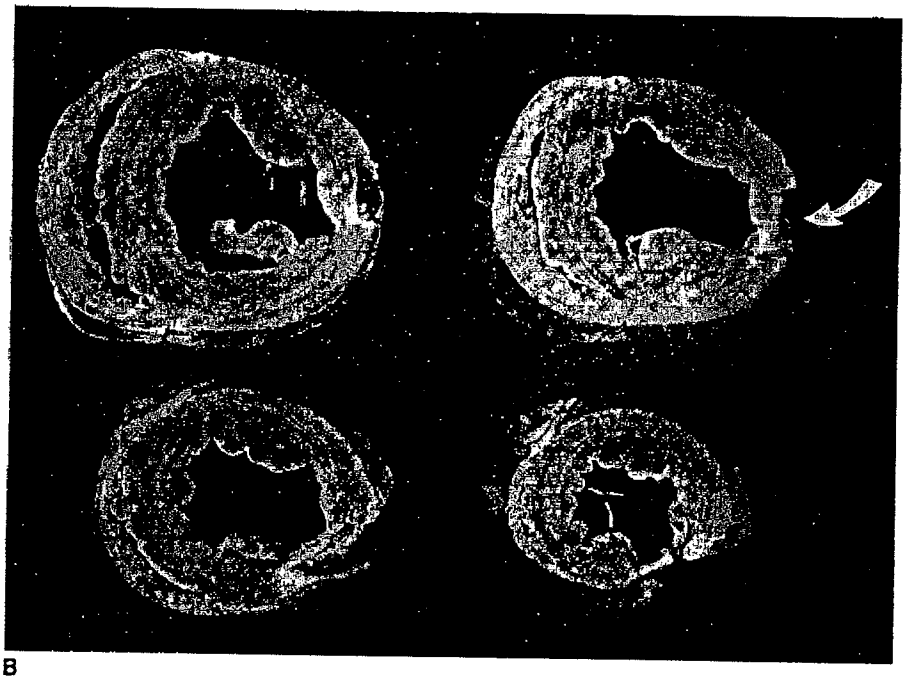
FIGURE 6. Panel A: *Photomicrograph of smooth muscle proliferative response in the center of a vessel with implanted stent. (Hematoxylin and eosin stain; original magnification, ×180).* Panel B: *Photomicrograph of immunohistochemical staining for actin contractile elements established that cells have smooth muscle cell morphology. (Original magnification, ×475.)*

dissection. Second, no evidence of medial dissection was seen at the time of final histological examination despite complete histological examination of all stented segments in all animals surviving to elective death 4–6 weeks after implantation.

Stent flexibility is a necessary prerequisite for successful intracoronary deployment because acute angles within the coronary tree must be negotiated. Delivery of metallic stents—even the articulated variant—is significantly limited by its lack of flexibility. The polymeric stent is highly flexible and not limited in this regard. In addition, a recent modification of the polymeric stent delivery system has enabled us to now use an over-the-wire delivery configuration.

PET (Dacron, Du Pont Co.) was chosen for initial study because of its widespread use in angioplasty

A



B

FIGURE 7. Panel A: *Photograph of large apical ventricular aneurysm that resulted from complete occlusion of the left anterior descending coronary artery.* Panel B: *Photograph of cross section of the left ventricle showing a healed transmural myocardial infarct (arrow) and replacement of ventricular muscle with fibrous tissue.*

balloon material and its ready availability. The biocompatibility of Dacron vascular grafts has been extensively studied.[8] After graft implantation, leukocytes (principally neutrophils and monocytes) migrate to the graft site. The leukocyte infiltration persists for several days to weeks; then, the neutrophils disappear. The monocytes may transform into tissue macrophages in an attempt to remove foreign graft material. Lymphocytes may also be seen but are not considered important in graft incorporation. The intimal proliferation elicited by PET stents was associated with both a pronounced chronic foreign body inflammatory reaction around the individual stent filaments and extensive smooth muscle proliferation. The development of marked neointimal

proliferation in response to a PET stent suggests a possible chemical as well as a mechanical stimulus for intimal proliferation after percutaneous transluminal coronary angioplasty (PTCA), possibly even from the PTCA balloon surface itself. Elucidation of the mechanism of PET-induced intimal proliferation may serve to explain certain aspects of the mechanism of human restenosis after angioplasty.

Animal Models of Postangioplasty Restenosis

Efforts to prevent human restenosis have been hampered by the lack of an accurate and easily reproducible animal model of neointimal proliferation.

This PET restenosis model is different from other models of restenosis in that neointimal formation is not induced by deep arterial injury and is unassociated with known lipid abnormalities. The architecture of the media of the vessel wall is preserved relatively intact with minimal apparent damage to the internal elastic lamina. Previously used animal models of restenosis have concentrated on the atheromatous nature of the experimental lesions,[9,10] on deep arterial injury, and on damage to the media. Sanborn et al[11] used rabbits fed atherogenic diets with serum cholesterol levels frequently exceeding 1,000 mg/dl. In addition to intimal thickening, the resulting atheromatous lesions of the aorta, iliac, and femoral vessels contained large numbers of foam cells, not typical of human restenotic tissue and not seen in our animals.

Steele et al[12] reported a model for restenosis using pig carotid arteries after endothelial denudation with the formation of thrombus and subsequent neointimal proliferation. Carotid and iliac arteries contain relatively more elastic fibers and proportionately less smooth muscle than coronary vessels and may be unsuitable as a model for human coronary restenosis because smooth muscle proliferation probably is a major factor in the genesis of the restenotic lesion.

The animals used in this study were fed a nonatherogenic diet. Histology resembling restenotic morphology without hyperlipidemia supports the concept that restenosis is a process independent of atherosclerosis. Although the proliferative effects might have been promoted further with a high-cholesterol diet, hyperlipidemia is clearly not a necessary condition for production of the proliferative response in this model. In this regard, it is important to emphasize that the microscopic coronary anatomy and intimal proliferation seen in these Yucatan swine were virtually identical to that previously described in human restenosis lesions.

Aneurysm Formation After Myocardial Infarction

Left ventricular aneurysm develops over a period of 2–8 weeks in 10–30% of patients after myocardial infarction.[13,14] Current experimental models of postinfarction ventricular aneurysm require a surgical approach with excision of a portion of the ventricular wall and the insertion of a Dacron patch to simulate a ventricular aneurysm.[15,16] Dacron is a multifilament yarn composed of small continuous filaments of PET. After implantation of a Dacron patch, a fibrin layer approximately ≤1 mm in thickness forms on the luminal surface of the graft. This is replaced within days to weeks by fibrous tissue that originates from the vasculature and grows through the interstices of the prosthesis.[17]

Such an experimental model has major theoretical and practical limitations. First, a thoracotomy with cardiopulmonary bypass is necessary with its attendant animal wastage. Second, insertion of a Dacron patch results in distortion of the normal ventricular anatomy. In contrast, in the pig model there is less anatomic distortion of the ventricle, with aneurysm formation due to loss of muscle with the resultant development of a full-thickness fibrotic scar in a manner similar to that of the postinfarct patient.

This model of ventricular aneurysm formation can be generated using regional anesthesia and sedation with a catheter-based technique and avoids the need for a thoracotomy and cardiopulmonary bypass. Ventricular aneurysms developed in all animals maintained for 4–6 weeks after stent implantation. The coronary anatomy of pigs is similar to that of humans in regard to histological structure, pattern of large vessel branching, and absence of significant preformed collateral vessels at the arterial level. The development of a relatively simple, inexpensive, and reproducible model for ventricular aneurysm after myocardial infarction is valuable and may aid the study of human postinfarction ventricular aneurysm formation.

Conclusions

We report the technical feasibility of percutaneous transluminal intracoronary deployment of polymeric stents. PET possesses the necessary mechanical properties for use as a stent but is unsuitable for human use because of the intense inflammatory and neointimal proliferative response elicited in this animal model. The PET model of coronary neointimal proliferation is unique and has not previously been reported.

We conclude that on the basis of immunohistochemical staining and transmission electron microscopy, vessel occlusion was due to smooth muscle cell neointimal proliferation. Polymeric stents, while having major potential advantages over metallic stents including biodegradability and drug-elution properties, may also be powerful inducers of neointimal proliferation. Although this study demonstrated that polymers can be configured in an appropriate design for intercoronary delivery, significant biomaterial problems exist before polymeric stents are ready for human investigation.

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Catheterization and Cardiovascular Interventions 57:266–271 (2002)

Basic Investigation

Estrogen-Eluting, Phosphorylcholine-Coated Stent Implantation Is Associated With Reduced Neointimal Formation But No Delay in Vascular Repair in a Porcine Coronary Model

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Estrogen can inhibit intimal proliferation and accelerate endothelial regeneration after angioplasty. This suggests that estrogen may prevent in-stent restenosis. Unlike other therapies to prevent restenosis, estrogen may also not delay endothelial regrowth, thereby avoiding the risk of late stent thrombosis. The purpose of this work was to determine the effect of a 17β-estradiol–eluting stent on neointimal formation in a porcine model. Each artery of six pigs was randomized to either a control, low-dose, or high-dose 17β-estradiol–eluting stent. All animals were sacrificed at 30 days for histopathological analysis. There was a 40% reduction in intimal area in the high-dose stents compared with control stents (2.54 ± 1.0 vs. 4.13 ± 1.1 mm², for high dose vs. control, respectively; $P < 0.05$). There was complete endothelial regeneration at 30 days and similar inflammatory response to stenting on histopathology in all the stent groups. This is the first study to show that 17β-estradiol–eluting stents are associated with reduced neointimal formation without affecting endothelial regeneration in the pig model of in-stent restenosis. Estrogen-coated stents may have a potential benefit in the prevention and treatment of in-stent restenosis. *Cathet Cardiovasc Intervent 2002;57:266–271.*

Key words: stents; restenosis; estrogen; drug-eluting stent; endothelium

INTRODUCTION

Restenosis remains the final frontier in interventional cardiology. Current treatments such as vascular brachytherapy are limited because of safety issues and the risk of late stent thrombosis [1]. A delay in reendothelialization causing a persistent thrombogenic coronary surface is the most plausible explanation for the latter. Local drug delivery to the site of vascular injury via a stent is a new alternative being tested for restenosis [2–4]. However, the stent coatings may theoretically delay endothelial regrowth and pose the same thrombosis risks as brachytherapy.

The female hormone estrogen has been shown to inhibit intimal smooth muscle cell proliferation and migration in animal models [5–10]. This suggests a protective effect on atherosclerosis and restenosis. However, unlike antiproliferative drugs such as rapamycin and paclitaxel, estrogen has been shown to accelerate reendothelialization in response to injury in experimental models [11,12]. It has been shown that the intracoronary infusion of

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estrogen can inhibit smooth muscle cell proliferation in the pig after angioplasty [13] without affecting endothelial regrowth and function [14]. The antiatherogenic properties of estrogen [15], along with its potential to inhibit neointimal proliferation without preventing endothelial repair, make it uniquely well suited as a stent coating. The objective of this study was to test the hypothesis that estrogen reduces neointimal formation without affecting endothelial repair.

MATERIALS AND METHODS

Animal Preparation

The experiment and animal care conformed to National Institutes of Health and American Heart Association guidelines for the care and use of animals and were approved by the Institutional Animal Care and Use Committee at the Washington Hospital Center. Six domestic juvenile swine weighing 35–45 kg were used. They were premedicated with acetylsalicyclic acid 350 mg for a day prior to and 75 mg of clopidogrel for 3 days prior to the procedure and until sacrifice. The procedure was performed under general anesthesia. The swines were sedated with a combination of ketamine (20 mg/kg) and xylazine (2 mg/kg) by intramuscular injection. They were given pentobarbital (10–30 mg/kg IV) and were subsequently intubated and ventilated with oxygen (2 L/min) and isoflurane 1% (1.5 L/min). An 8 Fr introducer sheath was inserted into the right carotid artery by surgical cutdown. Heparin (150 units/kg) was administered intra-arterially. Heart rate, blood pressure, and electrocardiography were monitored throughout the procedure.

Protocol for Loading of 17β-Estradiol Onto Stents

Two doses of 17β-estradiol powder (100 mg, dissolved in 5.0 ml sterile ethanol; Sigma, St. Louis, MO) were coated onto phosphorylcholine (PC)-coated stainless steel stents under sterile conditions in the cardiac catheterization laboratory (BiodivYsio DD Stent; 3.0 mm × 18 mm; Biocompatibles, Surrey, U.K.). The PC polymer is an inert and stable coating that via a sponge-like mechanism can absorb a drug into the polymer, subsequently to be eluted from the stent into the desired region of vessel over a prolonged period of time. The stents were immersed into the estradiol solution for 5 min and then allowed to dry at room temperature for another 5 min. For the high-dose stent, an additional 10 μl aliquot of solution was pipetted onto the stent. After being allowed to dry for 1 min, this step was repeated and the stent was allowed to dry for 10 min prior to implantation. In vitro studies indicate that an estradiol dose of 67 μg (range, 51–88 μg) for the low-dose stent and 240 μg (range, 229–254 μg) for the high-dose can be loaded onto a 3.0 × 18 mm stent.

Stent Deployment

Coronary angiography was performed after intracoronary nitroglycerin (200 μg) administration and recorded on cine film (Phillips Cardiodiagnost, Shelton, CT). Using high-pressure dilatation (12–14 atm × 30 sec), a single stent of each type was deployed in all three epicardial coronary arteries of each animal in a randomized fashion so that the three different types of stents were deployed in a different artery for each pig. The operator was blinded to the stent type being deployed. The stent artery ratio was kept between 1:1.3 and 1:1.2. All animals tolerated the stenting procedure and survived until 30 days, after which they were sacrificed and the hearts were perfusion-fixed.

Quantitative Histomorphometric Analysis

The histopathologist was blinded to the stent types in each artery. The stented segments were dissected and embedded in plastic by dehydration in graded ethanol, cleared with xylene, and infiltrated with methyl-methacrylate containing 0.2 Gm of benzoyl peroxide activator per 5 ml of acrylic. Plastic sections cut with the low-speed diamond wafer mounted to the Buehler Isomet saw (Buehler, Evanston, IL) were used for histomorphometric analysis. Measurements of the stented artery were carried out with a Nikon Labophot compound microscope equipped with 2× to 4× objectives. The visual field of the microscope was integrated to the LED-lit cursor of a standard digitizing pad through a Nikon drawing tube attachment with a 1.25× magnification factor. Measurements were carried out using Sigmascan morphometric software (Jandel Scientific, San Rafael, CA) with an Intel/Pentium-based personal computer system. Three areas delimited by the external elastic membrane, the internal elastic membranes (IEM) along with the inner surface of the stent, and the luminal surface were measured in square millimeters. Various dimensions were calculated off these three area measurements. In addition, semiquantitative scores were given for injury, inflammation, vascularization, fibrin, intimal smooth muscle, and adventitial fibrosis. The degree or severity of inflammation and vessel injury score was determined by the method described by Kornowski et al. [16]. The scores were based on degree and extent of injury. Injury score depends on the part of the arterial wall perforated by the device (degree score: 1, break in IEM; 2, break in media; 3, break in adventitia). Extent refers to fraction of circumference of artery involved. Inflammation score depends on the degree of inflammation and extent of the circumference of the artery involved (degree score: 1, scattered inflammatory cells; 2,

TABLE I. Histomorphometric Analysis

	Control	Low dose	High dose	P
Vessel area	9.69 ± 2.21	9.92 ± 2.04	10.45 ± 1.45	NS
Luminal area	3.49 ± 1.41	4.20 ± 1.74	5.4 ± 1.70	NS
Intimal area	4.13 ± 1.1	3.60 ± 0.79	2.54 ± 1.0	<0.05*
Stent strut area	7.61 ± 1.70	7.80 ± 1.53	8.01 ± 1.23	NS
Injury score	2.11 ± 0.47	2.19 ± 0.43	1.95 ± 0.61	NS
Intimal area/injury score	1.96 ± 0.32	1.66 ± 0.33	1.32 ± 0.40	<0.01*
Endothelialization score	3+	3+	3+	NS

*P value for control vs. high dose (ANOVA posttest with Bonferroni correction).

TABLE II. Histopathology Analysis

	Control	Low dose	High dose	P
Adventitial fibrosis	1.11 ± 0.56	1.25 ± 0.48	0.86 ± 0.88	NS
Intimal smooth muscle cell colonization	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	NS
Intimal fibrin deposition	0.45 ± 0.33	0.20 ± 0.25	0.09 ± 0.09	NS
Intimal vascularity	0.95 ± 0.83	0.78 ± 1.02	0.50 ± 1.07	NS
Inflammation score	1.36 ± 0.82	1.19 ± 0.89	1.12 ± 0.99	NS

aggregate around half of strut; 3, aggregate circumferentially around strut). The extent refers to the portion of the circumference of the artery involved. The extent of involvement is based on percent of the circumference of the arterial cross-section affected instead of average of all the struts in the cross-section.

Area measurements were obtained by tracing the external elastic lamina (vessel area, VA, mm^2), stent line (stent strut area, mm^2), lumen perimeter (luminal area, LA, mm^2), and neointimal perimeter (intimal area, IA, mm^2). Surface endothelialization was evaluated by light microscopy under 20× and 40× objectives. The scoring of endothelialization is based on percent of intimal surface covered by endothelial cells (1+, less than 1/4 of the intimal surface is covered by endothelial cells; 2+, over 1/4 and less than 3/4 covered; 3+, greater than 3/4 to complete coverage of the intimal surface).

Statistical Analysis

Data (mean ± standard deviation) were analyzed for overall differences between treatment groups using an ANOVA with a posthoc Bonferroni analysis. Comparison of the mean values with a P value of less than 0.05 was considered statistically significant.

RESULTS

The quantitative histomorphometry results of all three stent groups are shown in Table I. There were no differences in vessel area, luminal area, and stent strut area between the three groups. There was a 40% reduction in intimal area in the high-dose stents compared with control stents (2.54 ± 1.0 vs. 4.13 ± 1.1 mm^2, for high dose vs. control, respectively; $P < 0.05$; Table I). There were no differences in injury score; however, there was a reduction in the IA/injury score ratio in the high-dose group compared with the control stents (1.32 ± 0.40 vs. 1.96 ± 0.32 mm^2, for high dose vs. control, respectively; $P < 0.01$; Table I). Complete and equivalent healing and reendothelialization were seen in all groups. No cases of aneurysm formation or thrombosis were observed in any treatment group. The histopathology analysis of all three groups is shown in Table II. There were no differences in amount of adventitial fibrosis deposition, intimal smooth muscle cell colonization, intimal fibrin deposition, intimal vascularity, and in the inflammation score response to stent struts. Figure 1A illustrates the histological appearance of the control stented segments at 30 days, Figure 1B illustrates the histological appearance of the low-dose stented segments at 30 days, and Figure 1C illustrates the histological appearance of the high-dose stented segments at 30 days.

DISCUSSION

This is the first study to demonstrate that an estrogen-eluting stent is associated with inhibition of neointimal formation. There was a 40% reduction in neointimal formation between the high-dose 17β-estradiol stents and control stents. Importantly, there was complete endothelialization around the stent struts seen in all three groups at 30 days. There was also no difference in cellularity, fibrin deposition, and inflammatory response to the stents between the three groups. This suggests that estrogen may prevent restenosis and have a theoretical advantage over other antiproliferative agents, such as rapamycin or paclitaxel, or brachytherapy in that estrogen may not delay regrowth of the endothelium.

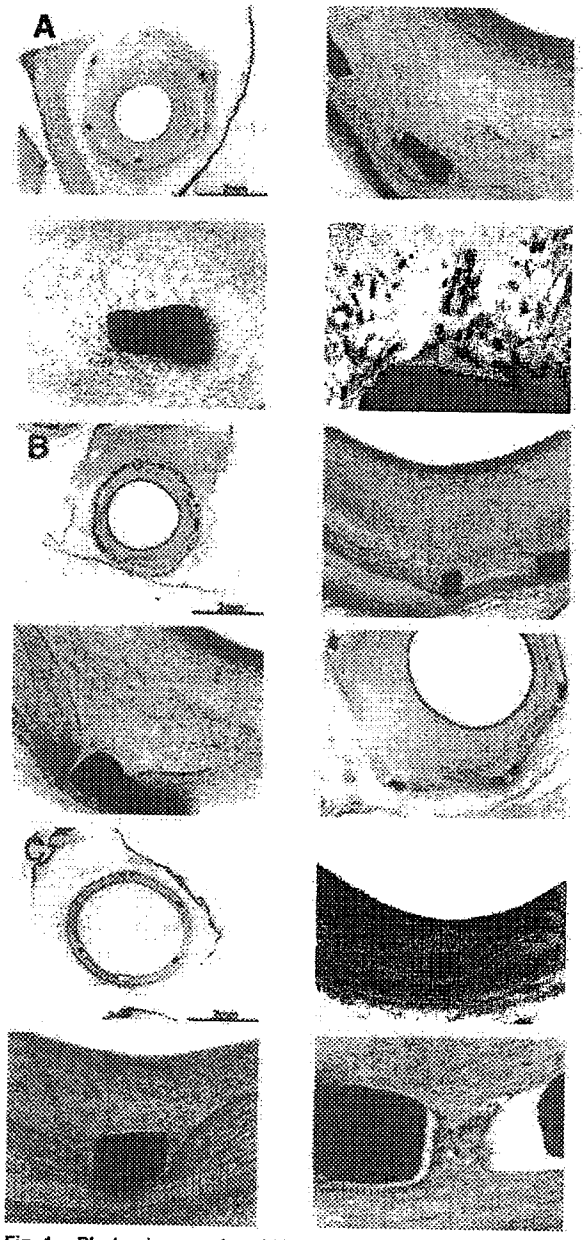

Fig. 1. Photomicrographs of histological sections 30 days after delivery of (A) control stent, (B) low-dose 17β-estradiol stent, and (C) high-dose 17β-estradiol stent illustrating degree of neointimal formation.

The sex hormone estrogen is known to have cardioprotective properties. Premenopausal women have a low incidence of coronary heart disease (CHD) [17]. Many epidemiological studies suggest that estrogen replacement therapy (ERT) may reduce the risk of CHD [18] and observational studies suggest that ERT may reduce the risk of restenosis or improve outcomes after angioplasty in postmenopausal women [19–22]. Unfortunately, randomized clinical trials have failed to prove a cardiac benefit with the use of oral ERT in postmenopausal women [23,24]. Potential reasons for this inconsistency include the opposing effects of progesterone used and the induction of a procoagulant state due to the first-pass effect of oral estrogens on the liver. The systemic use of this hormone is limited by the possible hyperplastic effects of estrogen on the uterus and breast in women [25], and also by the feminizing effects in males.

Several experimental and clinical studies have identified many athero-protective effects of estrogen [26]. Estrogen can inhibit plaque development in monkeys [27]. Estrogen can also act as an antioxidant, alter lipoproteins, coagulation, and fibrinolytic factors [15,26,28]. Estrogen has also been shown to inhibit smooth muscle cell proliferation and migration, the mechanism involved in in-stent restenosis [5–10]. This effect of estrogen is mediated via a specific estrogen receptor [7]. Additionally, estrogen can inhibit adventitial fibroblast migration, the cellular mechanism involved in unfavorable remodeling following balloon angioplasty [9]. Estrogen can attenuate abnormal vasoconstriction and platelet and leukocyte adhesion [26]. Finally, estrogen accelerates reendothelialization after balloon angioplasty in animal models [11,12,29].

Previous studies support an antirestenosis and endothelial protective effect of estrogen [13,30]. Chandrasekar and Tanguay [13] have shown that estrogen delivered to the coronary artery via an infusion catheter can inhibit smooth muscle cell proliferation in the pig following angioplasty. This same group has also demonstrated that estrogen enhances regrowth of the endothelium and preservation of endothelial function via the expression of eNOS [14].

The pathophysiology of restenosis involves neointimal hyperplasia and negative vessel remodeling [31]. Brachytherapy is the only current FDA-approved treatment for restenosis. However, the risk of late stent thrombosis, safety factors, and widespread availability limits its use [1]. Drug-eluting stents are currently under investigation as an alternative to prevent restenosis [2–4,32–34]. Typically, a drug is bound to a stent via a polymer. Polymers differ in their biocompatibility and pharmacokinetic properties and may account for the efficacy of the drug-coated stent in reducing restenosis [35]. Early clinical trials using different antiproliferative agents appear to be effective in the prevention of intimal hyperplasia [3,4]. Theoretically, late stent thrombosis may remain an issue as these agents may also retard regrowth of the

endothelium. Indeed, some malaposition has already been observed on intravascular ultrasound follow-up angiography in a randomized trial, although no clinical sequele have been noted [36]. Estrogen may avoid this problem.

In our study, we chose to deliver estrogen directly on a stent that has a biocompatible nondegradable PC polymer that has been shown to endothelialize normally and does not produce any adverse tissue reaction in animal models [37]. In addition, clinical studies with this PC-coated stent (without drug) have shown similar restenosis rates compared with noncoated stents [38]. Although the low-dose 17β-estradiol stents only demonstrated a trend toward a reduction in intimal area, the high-dose 17β-estradiol stents significantly reduced neointimal thickening by approximately 40% compared with control stents. This reduction is comparable to experimental studies with rapamycin and paclitaxel [4,39,40]. Another theoretical advantage noted in this study was that there was no evidence of inhibition of endothelial cell regeneration in the low- or high-dose stented arteries compared with control. In addition, there was no difference in intimal fibrin, intimal vascularity intimal smooth muscle cell content, adventitial fibrosis, inflammatory response, or any other features of a tissue reaction.

Study Limitations

The loading process of the estrogen onto the stent must be performed under sterile conditions and takes approximately 15 min. This was performed in the cardiac catheterization laboratory. Theoretical issues regarding potential risk of contamination of the drug and extra time for the procedure may be offset by the reduced costs associated with performing the loading process in the catheterization laboratory with an already approved stent. There is also the potential risk of the entire drug loaded on the stent eluting into the circulation as a bolus dose. However, the maximum amount of drug loaded on the stent (264 μg), is a relatively low systemic dose of estrogen. Systemic doses usually range between 25 and 30 μg/kg, which is more than 2 to 3 times the total dose of estrogen loaded onto the high-dose stent. Many clinical studies have acutely administered higher doses (systemically or intracoronary) in both male and female with no untoward effects [41–43]. Another limitation of this study is that we did not perform endothelial function studies or eNOS or estrogen receptor studies to explain the mechanisms of the effect of estrogen. This is the subject of ongoing work.

This is the first experimental study to demonstrate that estrogen-impregnated stents may reduce the neointimal formation with complete reendothelialization at 4 weeks. Since 17β-estradiol is an endogenous circulating hormone in both males and females, in this relatively low systemic dose it may provide a simple, benign, nontoxic therapy for the prevention of restenosis. Clinical studies will soon commence and hopefully corroborate this potential benefit of estrogen.

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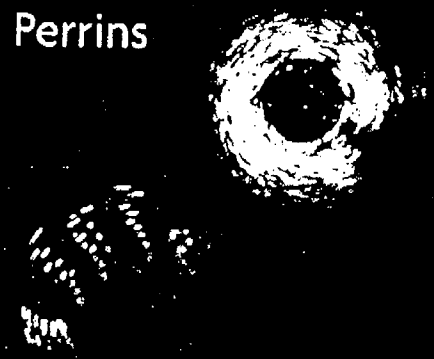

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